IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Chong-Sheng YUAN

Application No.: 10/665,883 Confirmation No.: 6779

Filed: September 19, 2003 Art Unit: 1652

For: DETERMINATION OF IONS USING ION-SENSITIVE ENZYMES Examiner: C. Patterson

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Chong-Cheng Yuan, declare as follows:

1. I am the named inventor for the patent application identified above. I have reviewed the application and am familiar with its contents. A copy of my *curriculum vitae* is attached as **Exhibit C**.

2. I understand that the claims currently under examination relate to a method for assaying a sample to determine its content of sodium ions or of lithium ions, using a sodium-sensitive or a lithium-sensitive bisphosphate nucleotidase.

3. I understand that the Patent Examiner rejected the pending claims because the specification allegedly failed to state which specific enzymes could be used in the claimed methods to detect either sodium or lithium.

4. The application cites numerous references that describe suitable peptides for practicing the claimed methods. Some of the cited references specify the sensitivity of those peptides to either sodium or lithium or both. From my experience, I believe one of ordinary skill would have been able to use these cited references to identify suitable peptides for use in the claimed methods to assay either sodium or lithium, and would therefore have been able to practice the invention based on the specification as filed.

5. The specification discloses that a peptide as described in Section B of the specification was successfully used to assay the sodium ion content of a solution, and exhibited an IC50 of 20 mM (see paragraph [00112]). The specification further discloses that a peptide as described in Section B of the specification was successfully used to assay the lithium ion content of a solution, and exhibited an ID50 of 0.10 mM (see paragraph [00122]).

6. From my personal knowledge of the assay used to generate the data included in the present application, I state that the assay for sodium ions and the assay for lithium ions identified in the preceding paragraph were both run with the same nucleotidase enzyme. I further state that the enzyme used in both of those assays was a chimeric protein having the amino acid sequence of SEQ ID NO: 4, which is disclosed at paragraph [0050] in the application as a suitable protein for such assays.

7. I understand that the Examiner also indicated that a particular enzyme would need to be selective for either sodium or lithium to be used in the claimed methods.

8. The method of the claimed invention can be used successfully to assess the level of sodium in a sample known to have a low level of lithium, or to assess the level of lithium in a sample known to have a low level of sodium; and the method can be used to assess the level of either sodium or lithium if the level of the other is known by a separate method.

9. Most samples of biological significance contain very low levels of lithium, unless the subject providing the sample is being treated with a lithium-containing medication. Most human samples contain a predictable level of sodium, since its concentration is usually closely regulated and thus falls within a narrow range.

10. In view of these known characteristics, the methods of the invention can be used to determine the level of sodium or lithium in many samples even if the nucleotidase enzyme used in the assay is sensitive to both sodium and lithium ions, provided the sensitivity of the protein to each of the two ions is known.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at San Diego (city), CA (state), on 19 (day) June (month) 2006.

Chongsheng Yuan

Chong-Sheng Yuan

Curriculum Vita

Chong S. Yuan

Personal

Birthdate:	January 11, 1959
Birthplace:	Zhejiang, China
Marital Status:	Married
Citizenship:	U.S.A.
Home address:	3590 Torrey View CT, San Diego, CA 92130
Phone numbers:	858-792-7866 (H) 858-455-4760 (O)

Academic Training and Degrees

Institution	Degree	Years	Field
University of Ningbo, Zhejiang, China	B. S.	1978–1982	Food Chemistry
Ocean University of China, Qingdao, China	Graduate school	1983-1984	Food Chemistry
University of Mie, Japan	M. S.	1984–1987	Biochemistry Enzymology
University of Kyushu, Japan	Ph. D.	1987–1990	Biochemistry Enzymology
University of Kansas	Post-doctoral fellow	1991–1995	Pharmaceutical Chemistry

Employment

Institution	Title	Duration	Field
University of Kansas	Res. Assistant Professor	1995-1997	Biochemistry Drug Design
Tanabe Res. Lab, Inc.	Senior Scientist/Group Leader	1997-2000	Drug Design Biology
Diazyme Laboratories Division, General Atomics	Managing Director	2000-present	Clinical Diagnostics, Pharmaceutics

Research Activities

1986-1990 Ph.D. thesis under the guidance of Prof. Manabu Kitamikado in the Department of Agriculture, University of Kyushu, Japan

Research focused on the purification, enzymatic and immunological characterizations of novel glucosaminidases involved in the lysosomal storage disease, G_{m2}-gangliosidosis.

1991-1995 Post-doctoral fellow with Prof. Ronald Borchardt in the Department of Pharmaceuticals Chemistry, University of Kansas, USA.

Research focused on the discovery of broad-spectrum antiviral agents targeting S-adenosylhomocysteine hydrolase. Extensively studied the mechanism of S-adenosylhomocysteine hydrolase and discovered a new enzyme reaction mechanism that can be utilized for design of novel inhibitors of the enzyme.

1995-1997 Research Assistant Professor in Higuchi Biosciences Center, University of Kansas, U.S.A.

Research focused on anticancer drug development from natural products and on the development of drugs lowering plasma level of homocysteine as a treatment for cardiovascular diseases.

1997-2000 Senior Scientist/Group leader in the Department of Biology, Tanabe Research Laboratories, Inc., San Diego, CA, USA.

Research focused on the discovery of novel immunosuppressants for organ transplantation. Discovered a new mechanism for T-cell inactivation and inhibition of T-cell proliferation.

2000-present Co-founder and Managing Director of Diazyme Laboratories, Division of General Atomics. San Diego, CA, USA.

Research focused on the development of novel enzyme-based clinical diagnostic reagents, and enzyme inhibitors as novel immunosuppressants. Executive management of strategic planning and international marketing.

Honors and Awards

Chinese Government Scholarship for Outstanding Graduate Student to Study Abroad (1984 to 1990).
Traineeship Award from Japanese Association for Overseas Technology Scholarship (1990 to 1991).
Invited speaker at FASEB summer research conference on biological methylation, 1997, Vermont.

Memberships in Professional Organizations

Full member of American Society for Biochemistry and Molecular Biology
Full member of American Association of Clinical Chemistry
Full member of American Association of Pharmaceutical Sciences

Independent Research Grant Awards

Research grant from Beso Biological Engineering R & D Inc., August 1995 to July 1997, $89,000 over 2 years. This grant is supporting research in anticancer drug discovery.

Management training

BioCom's Industrial Management and Leadership Training Course, 1998

List of Publications

1 Dou, C. Xia D.Y. Zhang, L.Q. Chen, X.R. Flores, P. Datta, A. and Yuan, C. S. Development of a Novel Enzymatic Cycling Assay for Total homocysteine (2005) Clinical Chemistry, 51: 1987-1989

2. Dou. C. Aleshin, O. Datta, A. and Yuan, C.S. Automated Enzymatic Assay for Measurement of Lithium Ions in Human Serum (2005) Clinical Chemistry, 51: 1989-1991

3. Yang, Y. Dou, C. Yuan, C.S. and Datta A. Development of an Automated Enzymatic Assay for the Determination of Glycated Serum Protein in Human Serum (2005), 51: 1991-1992

4. Wu, Q.L., et al. Yuan, C.S. and Zuo, J. P. Inhibition of S-Adenosylhomocysteine Hydrolase Induces Immunosuppression (2005) JPET, 313: 1-7

5. Saso, Y, Conner, E., Teegarden, B. and Yuan, C.S. S-Adenosyl-L-homocysteine Hydrolase Inhibitor mediates Immunosuppressive Effects in Vivo: Suppression of Delayed Type Hypersensitivity Ear Swelling and Peptidoglycan Polysaccharide-Induced Arthritis (2001) *JPET*, 296: 106-112

6. Yuan, C. S., Robins, M. J., and Borchardt, R. T. Recent Advances in S-Adenosylhomocysteine Hydrolase Inhibitors and Their Potential Clinical Applications (1999) in *Expert Opinion on Therapeutic Patents*, 9: 1197-1206

7. Turner, M. A., Yuan, C. S., Borchardt, R. T., Hershfield, M. S., Smith, G. D., and Howell, P. L. Structure of Human Placental S-Adenosylhomocysteine Hydrolase: Determination of a 30 Selenium Atom Substructure (1998) *Nature Structural Biology* 5, 369-376

8. Yuan, C.S. Wnuk, S. F., Robins, M., and Borchardt, R. T. A Novel Mechanism-Based Inhibitor That Covalently Modifies The Active-Site of Human Placental *S*-Adenosylhomocysteine Hydrolase (1998) *J. Biol. Chem.* 273, 18191-18197

9. Wnuk, S. F., Mao Y., Yuan, C. S., Borchardt, T. R., Andrei, G., Balzarini, J., De Clercq, E., and Robins, M. J. Discovery of type II (covalent) inactivation of S-adenosylhomocysteine hydrolase involving its "hydrolytic actaivity": Synthesis and evaluation of dihalohomovinyl nucleoside analogues derived from adenosine (1998) *J. Med. Chem.* 41, 3078-3083

10. Robins, M. J., Wnuk, S. F., Yang, X., Yuan, C. S., Borchardt, T. R., Balzarini, J., and De Clercq, E. Inactivation of S-adenosylhomocysteine hydrolase and antiviral activity with 5',5',6',6'-tetradehydro-6'-deoxy-6'-halohomoadenosine analogues (4'-haloacetylene analogues derived from adenosine) (1998) *J. Med. Chem.* 41, 3867-3864

11. Robins, M. J., Neschadimenko, V., Ro, B., Yuan, C. S., Borchardt, T. R., and Wnuk, S. F. Nucleic acid related compounds. 101. S-adenosylhomocysteine hydrolase does not hydrate (5'-fluoro)vinyl or (6'-halo)homovinyl analogues derived from 3'-deoxadenosine or 3'-(chloro or fluoro)-3'-deoxyadenosine (1998) *J. Org. Chem. 63*, 1205-1211

12. Wnuk, S. F., Yuan, C. S., Borchardt, R. T., and Robins, M. Synthesis of homologated halovinyl derivatives from aristeromycin and their inhibition of human placental S-adenosylhomocysteine hydrolase (1998) *Nucleosides and Nucleotides* 17, 99-113

13. Wnuk, S. F., Yuan, C. S., Borchardt, T. R., Balzarini, J., De Clercq, E., and Robins, M. J. Anticancer and Antiviral effects and inactaivation of S-adenosylhomocysteine hydrolase with 5'-carboxaldhydes and oximes synthesized from adenosine and sugar-modifired analogues (1997) *J. Med. Chem.* 40, 1608-1618

14. Turner, M. A., Dole, K., Yuan, C. S., Hershfield, M. S., Borchardt, R. T., and Howell, P. L. Crystallization and Preliminary X-ray Analysis of Human Placental *S*-Adenosylhomocysteine Hydrolase (1997) *Acta Cryst.*, D53,339-341

15. Huang, H. Yuan, C. S., and Borchardt, R. T. Effect of Limited Proteolysis on the Stability and Enzymatic Function of *S*-Adenosylhomocysteine Hydrolase (1997) *J. Protein Science* 6, 1482-1490

16. Robins, M. J., Wnuk, S. F., Yuan, C. S., Borchardt, R. T., Balzarini, J., and De Clercq, E. Anticancer and Antiviral Effects and Inactivation of *S*-Adenosylhomocysteine Hydrolase with 5'-Carboxaldehydes and Oximes Synthesized from Adenosine and Sugar-Modified Analogues (1997) *J. Med. Chem.* 40, 1606-1618

17. Huang, H., Yuan, C. S., Wnuk, S. F., Robins, M., and Borchardt, R. T. the Mechanism of Inactivation of Human Placental *S*-Adenosylhomocysteine Hydrolase by (E)4',5'-Didehydro-5'-methoxyadenosine (DMAO) and Adenosine 5'-Carboxaldehyde Oxime (ACAO) (1997) *Biochim. Biophys. Acta.* 343, 109-117

18. Liu, S. Yuan, C. S., and Borchardt, R. T. Aristeromycin-5'-carboxaldehyde: A Potent Inhibitor of S-Adenosylhomocysteine Hydrolase (1996) *J. Med. Chem.* 39, 2347–2353

19. Yuan, C. S. , Ault-Riche, D. B., and Borchardt, R. T. Chemical Modification and Site-Directed Mutagenesis of Cysteine Residues in Human Placental S-Adenosylhomocysteine Hydrolase (1996) *J. Biol. Chem.* 271, 28009–28016

20. Yuan, C. S., Liu, S., Wnuk, S. F., Robins, M. J., and Borchardt, R. T. Design and Synthesis of *S*-Adenosylhomocysteine Hydrolase Inhibitors as Broad-Spectrum Antiviral Agents. In *Advances in Antiviral Drug Design* (E De Clercq, Ed.) JAI Press Inc. Volume. 2, (1996) pp. 41–88

21. Yuan, C. S., and Borchardt, R. T. Photoaffinity Labeling of *S*-Adenosylhomocysteine Hydrolase with [2-^{3}H]8-Azido-Adenosine (1995) *J. Biol. Chem.* 270, 16140–16146

22. Nicolaou, M. G., Yuan, C. S., and Borchardt, R. T. Phosphate Prodrugs for Amines Utilizing a Fast Intramolecular Hydroxyamide Lactonization (1996) *J. Org. Chem.*, 61, 8636-8641

23. Wnuk, S. F., Liu, S., Yuan, C. S., Borchardt, R. T., and Robins, M. J. Inactivation of S-Adenosylhomocysteine Hydrolase by Amide and Ester Derivatives of Adenosine 5'-Carboxylic Acid (1996) *J. Med. Chem.* 39, 4162–4166.

24. Gupta, R., Yuan, C. S., Ault-Riché, D. B., and Borchardt, T. R. Limited Proteolysis of *S*-Adenosylhomocysteine Hydrolase. Implications for Three-Dimensional Structure. (1995) *Arch. Biochem. Biophys.* 319, 365–371.

25. Yuan, C. S., Liu, S., Wnuk, S. F., Robins, M. J., and Borchardt, R. T. Rational Approaches to the Design of Mechanism-Based Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase (1995) *Nucleosides & Nucleotides* 14, 439–447

26. Jeong, L. S., Marquez, V., Yuan, C. S., and Borchardt, R. T. 4'1'a-Methanocarboxcyclic Adenosine Analogues as Potential Inhibitors of S-Adenosylhomocysteine Hydrolase (1995) *Heterocycles,* 41, 2651–2656

27. Yuan, C.S., Wnuk, S.F., Liu, S., Robins, M. J., and Borchardt, R.T. (*E*)-5',6'-Didehydro-6'-Deoxy-6'-Fluoro-Homoadenosine: A Substrate that Measures the Hydrolytic Activity of *S*-Adenosylhomocysteine Hydrolase (1994) *Biochemistry* 33, 12305–12311.

28. Ault-Riche, B., Yuan, C. S., and Borchardt, R. T. A Single Mutation at Lysine 426 of *S*-Adenosylhomocysteine Hydrolase Inactivates the Enzyme by Destabilizing the Enzyme's Quaternary Structure (1994) *J. Biol. Chem.* 269, 31472–31478.

29. Wnuk, S. F., Yuan, C. S., Borchardt, R. T., Balzarini, J., De Clercq, E., and Robins, M. J. Nucleic Acid Related Compounds. 84. Synthesis of 6'(*E* and *Z*)-Halogenohomovinyl Derivatives of Adenosine, their Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase, and their Anticancer/Antiviral Potencies with AdoHcy Hydrolase Inhibition (1994) *J. Med. Chem.* 37, 3579–3587.

30. Yuan, C. S., Liu, S., Wnuk, S. F., Robins, M. J., and Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosylhomocysteine Hydrolase by (*E*)-5',6'-Didehydro-6'-deoxy-6'-halo-homoadenosines (1994) *Biochemistry* 33, 3758–3756.

31. Yuan, C.S., Yeh, J., Liu, S., and Borchardt, R. T. Mechanism of Inhibition of *S*-Adenosylhomocysteine Hydrolase by (*Z*)-4',5'-Didehydro-5'-deoxy-5'-fluoroadenosine (1993) *J. Biol. Chem.* 268, 17030–17037.

32. Yuan, C. S., Yeh, J., Squier, T. C., Rawitch, A., and Borchardt, R. T. Ligand-Dependent Changes in Intrinsic Fluorescence of *S*-Adenosylhomocysteine Hydrolase: Implication for the Mechanism of Inhibitor-Induced Inhibition (1993) *Biochemistry* 32, 10414–0422.

33. Liu, S., Wnuk, S. F., Yuan, C. S., Robins, M., and Borchardt, R. T. Adenosine 5'-carboxaldehyde: A Potent Inhibitor of *S*-Adenosyl-L-homocysteine Hydrolase (1993) *J. Med. Chem.* 36, 883–887.

34. Robins, M. J., Wnuk, S. F., Mullah, K., Dalley, N. K., Yuan, C. S., Lee, Y., and Borchardt, R. T. Nucleic Acid Related Compounds. 80. Synthesis of 5'-*S*-(Alkyl and Aryl)-5'-fluoro-5'-thioadenosine with Xenon Difluoride or (Diethylamine)sulfur Trifluoride, Hydrolysis in Aqueous Buffers, and Inhibitions of *S*-Adenosyl-L-homocysteine Hydrolase by Derived "Adenosine 5'-Aldehyde" Species (1993) *J. Org. Chem.* 59, 544–555.

35. Robins, M. J., Wnuk, S. F., Mullah, K. B., Dalley, N. K., Borchardt, R. T., Lee, Y., and Yuan, C. S. Adenosine-Derived 5'- a-Halo Thioethers, Sulfoxide, Sulfone, and (5'-Halo)Methylene Analogues. Inhibition of *S*-Adenosyl-L-Homocysteine Hydrolase. In *Nucleoside and Nucleotides as Antitumor and Antiviral Agents* (Chu, C. K., and Baker, D. C., Eds). Plenum Press, New York, pp. 115–125, (1993).

36. Ault-Riche, D. B., Lee, Y., Yuan, C. S., Hasobe, M., Wolf, M. S., Borcherding, D. R., and Borchardt, R. T. Effects of 4'-Modified Analogs of Aristeromycin on the Metabolism of *S*-Adenosyl-L-homocysteine in Murine L-929 Cells (1993) *Mol. Pharma.* 43, 989–997.

37. Liu, S., Wolfe, M. S., Yuan, C. S., Ali, S. M., and Borchardt, R. T. Synthesis and Evaluation of 4',5'-Didehydro-5'-fluoroaristeromycin as Inhibitor of *S*-Adenosyl-L-homocysteine Hydrolase (1992) *Bioorg. Med. Chem. Lett.* 2, 1741–1744.

38. Robins, M. J., Samano, V., Zhang, W., Balzarini, J., De Clercq, E., Borchardt, R. T., Lee, Y., and Yuan, C. S. Synthesis and Biological Activity of 2'(and 3')-Deoxy-2'-(and 3')-methylenenucleoside Analogues that Function as Mechanism-Based Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase and/or Ribonucleotide Reductase (1992) *J. Med. Chem.* 35, 2283–2293.

39. Ueno, R. and Yuan, C. S. Neutral b-*N*-Acetylglucosaminidase from Carp Blood (1991) *Biochim. Biophys. Acta.* 1704, 79–84.

40. Yuan, C. S., Kitamikado, M., Yamaguchi, K., and Ueno, R. Oligosaccharase Activity of Neutral b-*N*-Acetylglucosaminidase from Carp Blood (1991) *Nippon Suisan Gakkaishi* 57, 325–328.

41. Kitamikado, M., Yuan, C. S., and Ueno, R. An Enzymatic Method Designed to Differentiate between Fresh and Frozen-Thawed Fish. *J. Food Sci.* (1990) 55, 74–76.

42. Ueno, R., Yu, X., and Yuan, C. S. Purification and Characterization of Two Acidic b-*N*-Acetylhexosaminidases from Carp Blood (1989) *Nippon Suisan Gakkaishi* 55, 1407–1413.

43. Ueno, R., Yuan, C. S., and Horiguchi, H. Distribution of b-*N*-Acetylglucosaminidase in Fish Blood (1988) *Nippon Suisan Gakkaishi* 54, 95–101.

44. Ueno, R., Yuan, C. S., and Horiguchi, H. Characterization of Neutral b-*N*-Acetylglucosaminidase from Carp Blood (1987) *Nippon Suisan Gakkaishi* 53, 1017–1024.

45. Ueno, R., Yuan, C. S., and Horiguchi, H. Purification of Neutral b-*N*-Acetylglucosaminidase from Carp Blood (1987) *Nippon Suisan Gakkaishi* 53, 1009–1016.